

SEQ ID No. 1:
Human RANKL
(aa 68)
FYFRAQMDPNRISEDTGTHCIYRILRLHENADFDQTTLTLESQDTKLIPDSCRRIKQAFQGAQKELQH
IVGSGHTRAERKAMVDGSLDLAKRSKLEAQPFAHLTINATDIPSGSHKVSLSWYHDRGWAKISNM
TFNGKLIYNQDGFYILYANICFRHHETSGDLATEYLQLMVYVTKTSIKIPSSHTLMKGGSTKYWS
GNSEFFHYFINVGGFKLRSGEETSI EYVSNPSLLDPDQDATYFGAFKVRDID
Human RANKL
(aa 317)

Figure 1a

SEQ ID No. 2:
TEV Protease Human RANKL
His Tag Cleavage Site (aa 159)
MGHHHHSSGLEVLFGQPKLEAQPFAHLTINATDIPSGSHKVSLSWYHDRGWAKISNMTF
SNGKLIYNQDGFYILYANICFRHHETSGDLATEYLQLMVYVTKTSIKIPSSHTLMKGGSTKY
WSGNSEFFHYFINVGGFKLRSGEETSI EYVSNPSLLDPDQDATYFGA-KVRDID
Human RANKL
(aa 317)

Figure 1b

SEQ ID No. 3:
Mouse RANKL
(aa 68)
FLYFRAQMDPNRISEDTGTHCIYRILRLHENADLQDSTLESQDTKLIPDSCRRMKQAFQGAQKE
LQHTVGQRFSGAPAMMEGSLDVAQRGKPEAQPFAHLTINAASTPSGSHKVTLSWYHDRG
WAKISNMTLSNGKLRVNQDGFYILYANICFRHHETSGVPTDYQLMVYVTKTSIKIPSSHN
LMKGGSTKNWSGNSEFFHYFINVGGFKLRSGEETSIQVSNPSLLDPDQDATYFGAFKQDI
D (Mouse RANKL aa 316)

Figure 1c

Human: 159 KLEAQPFPAHLTINATDIPSGSHKVSLSWYHDRGWAKISNMTFSNGKLI VNQDGFYYLYA 218
K EAQPFPAHLTINA IPSGSHKV+LSSWYHDRGWAKISNMT SNGKL VNQDGFYYLYA
Mouse: 158 KPEAQPFPAHLTINAASIPSGSHKVTLSSWYHDRGWAKISNMTLSNGKLRVNQDGFYYLYA 217

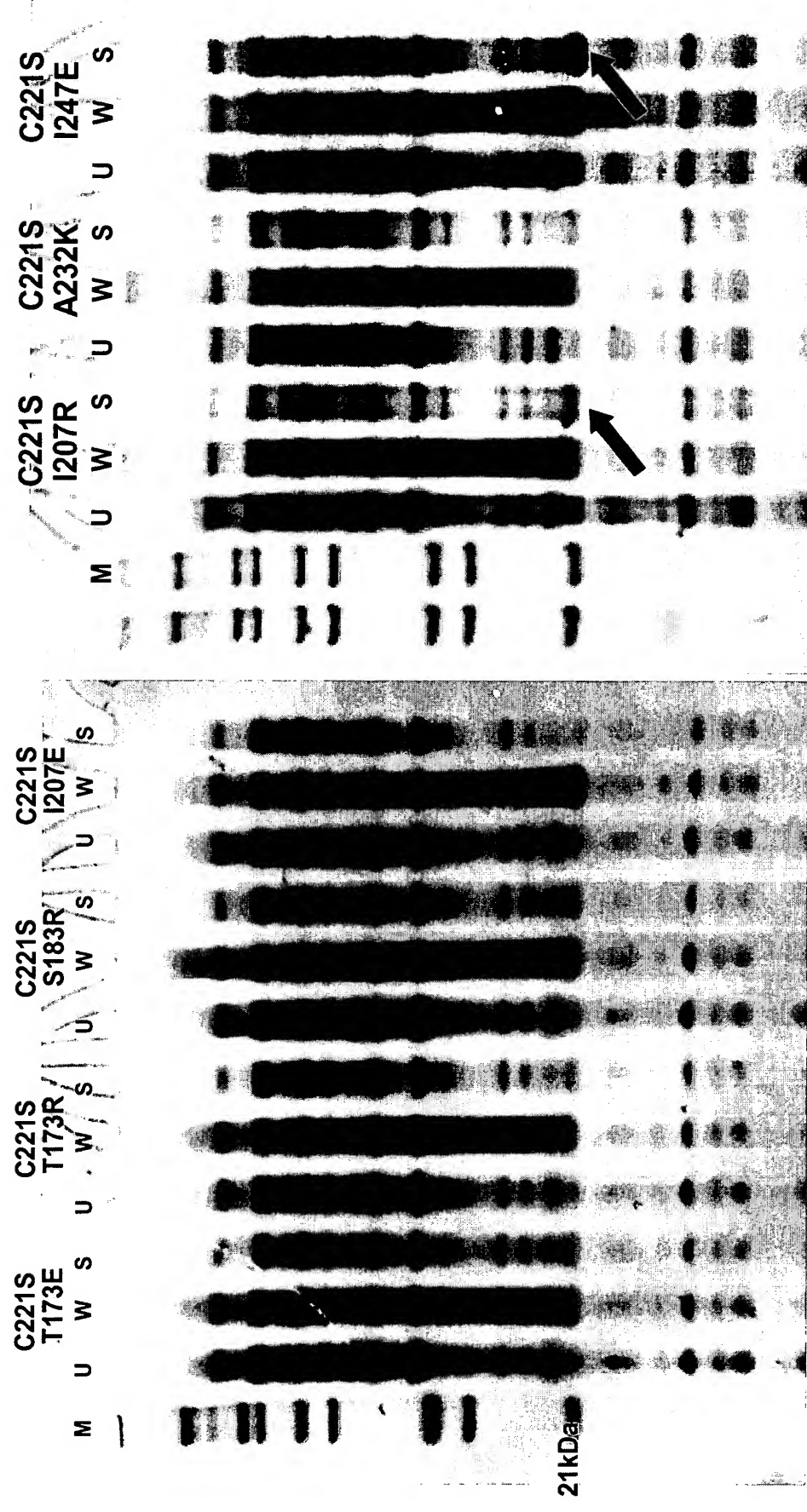
Human: 219 NICFRHHETSGDLATEYLQLMVYVTKTSIKIPSSHTLMKGGSTKYWSGNSEFFHFYSINVG 278
NICFRHHETSG + T+YLQLMVYV KTSIKIPSSH LMKGGSTK WSGNSEFFHFYSINVG
Mouse: 218 NICFRHHETSGSVPTDYLQLMVYVVKTSIKIPSSHNL MKGGSTKNWSGNSEFFHFYSINVG 277

Human: 279 GFFKLRSGEEISIEVSNPSLLDPDQDATYFGAFKVRDID 317
GFFKLR+GEEISI+VSNPSLLDPDQDATYFGAFKV+DID
Mouse: 278 GFFKL RAGEEISIQVSNPSLLDPDQDATYFGAFKVQDID 316

Figure 2a

T173R/C221S
T173E/C221S
S183R/C221S
I207D/C221S
I207E/C221S
I207K/C221S
I207R/C221S
A232K/C221S
I247A/C221S
I247D/C221S
I247E/C221S
I247K/C221S
I247Q/C221S
I247R/C221S
C221S/T173R/S183R/I207R/I247R

Figure 2b



Coomassie Stained Gels

Figure 3

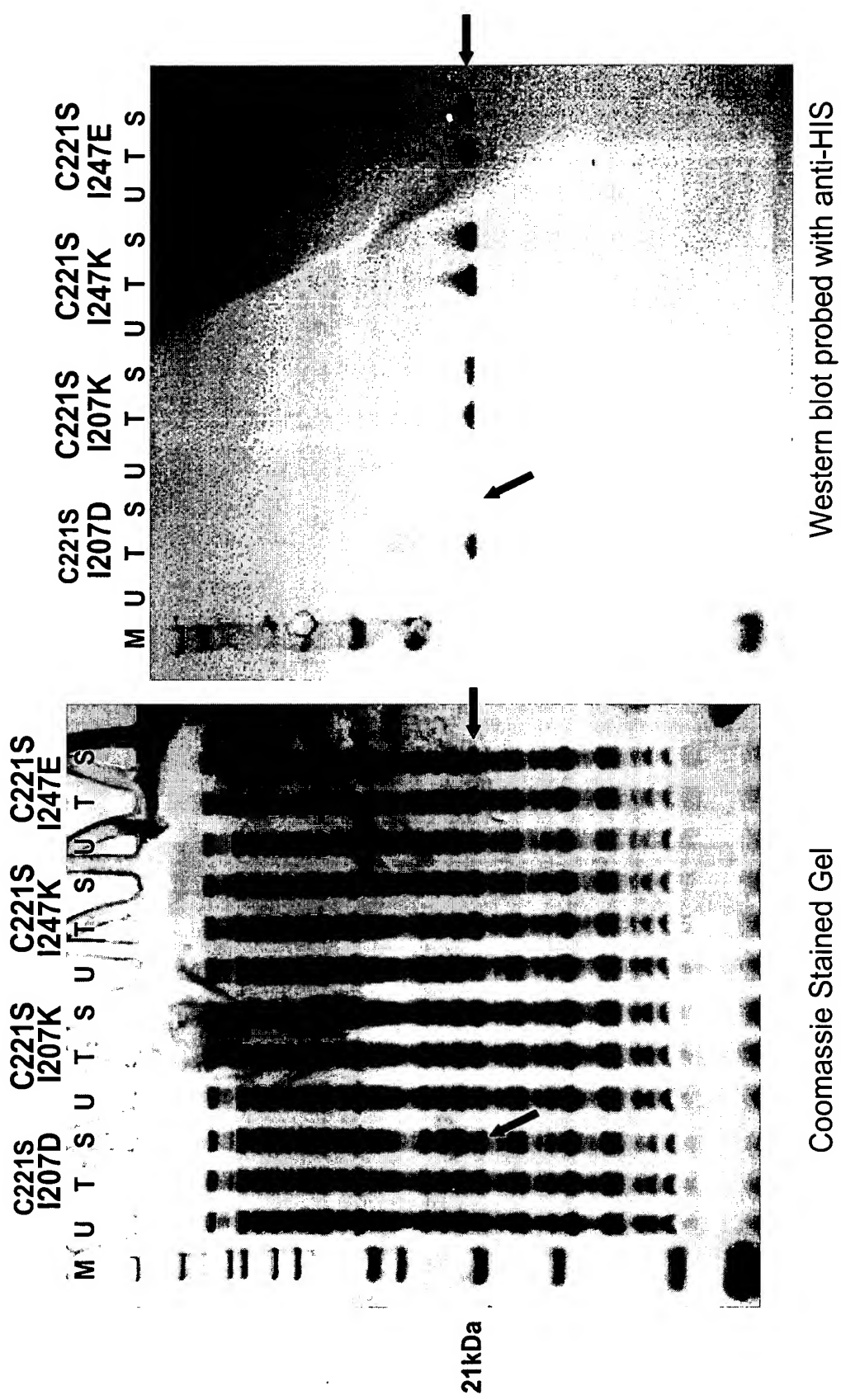
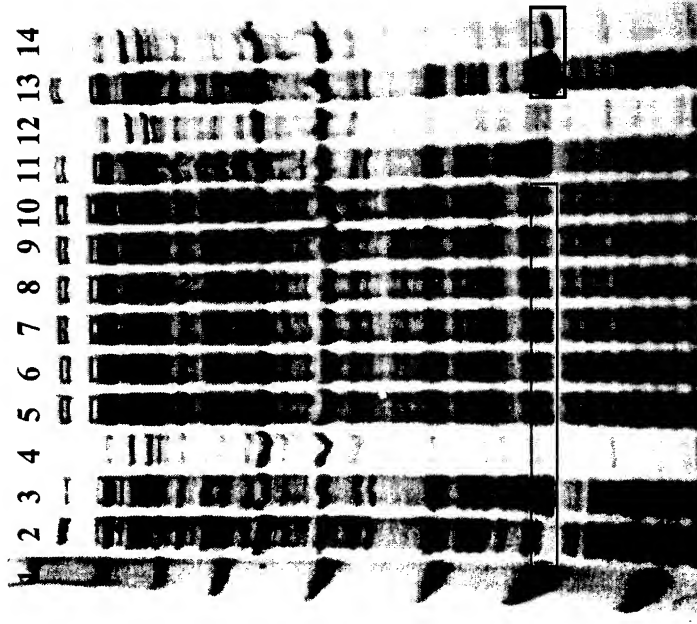
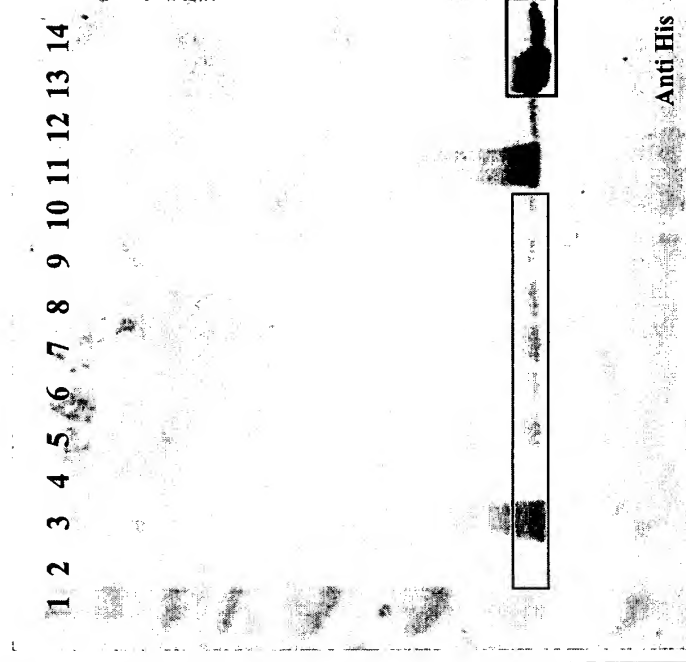


Figure 4



25C, 0.5 mM IPTG, 4 hr induction
Coomassie Stained Gel

Lane	Description	Strain
1	SeeBlue Plus2 MW Standards	NA
2	Pre Induced Total Protein	His-OPGL
3	Post Induced Total Protein	His-OPGL
4	Post Induced Soluble Protein	His-OPGL
5	Post Induced Total Protein	T173R/C221S
6	Post Induced Soluble Protein	T173R/C221S
7	Post Induced Total Protein	S183R/C221S
8	Post Induced Soluble Protein	S183R/C221S
9	Post Induced Total Protein	A232K/C221S
10	Post Induced Soluble Protein	A232K/C221S
11	Post Induced Total Protein	I207K/C221S
12	Post Induced Soluble Protein	I207K/C221S
13	Post Induced Total Protein	I247E/C221S
14	Post Induced Soluble Protein	I247E/C221S



Western blot probed with anti-HIS

Figure 5

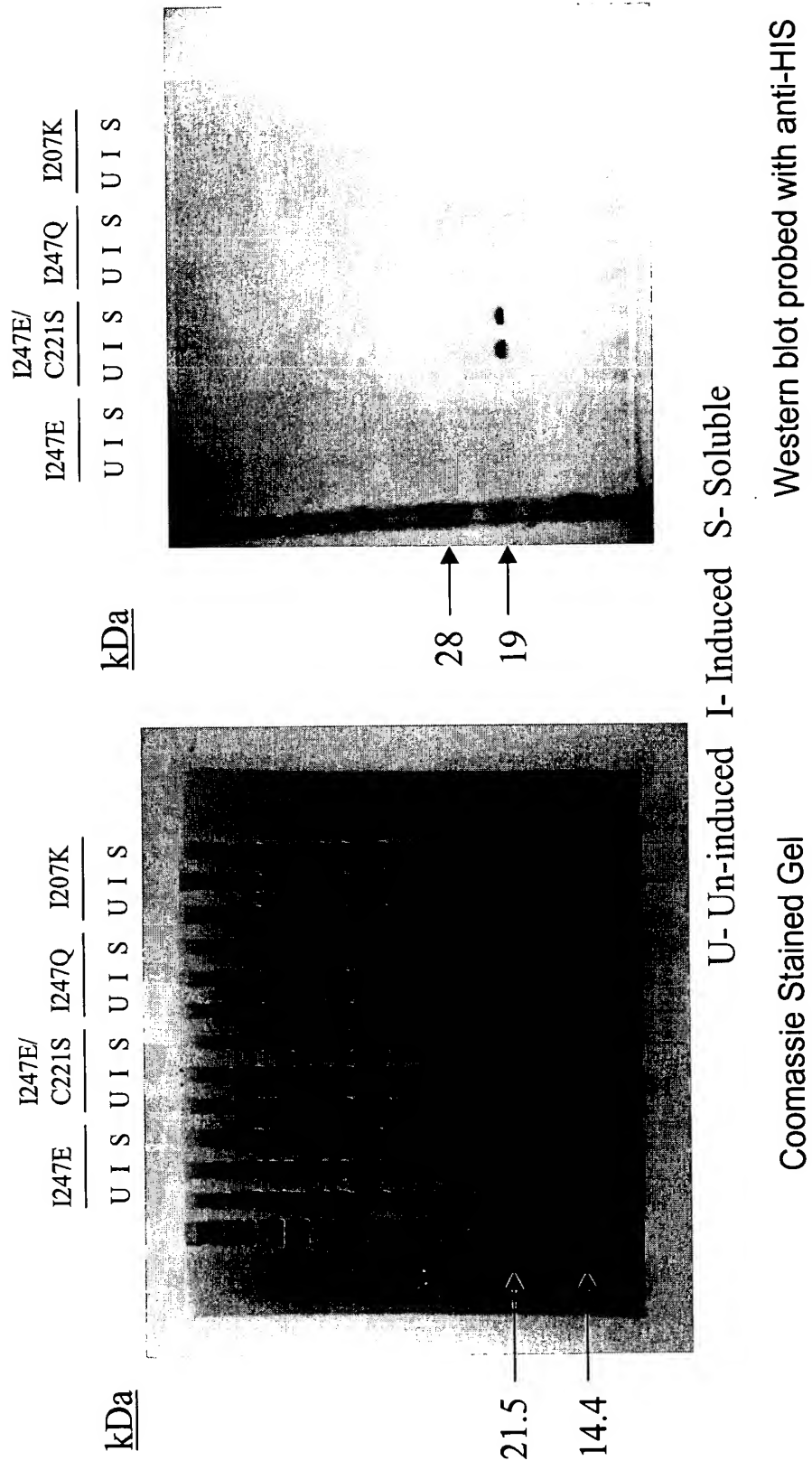


Figure 6

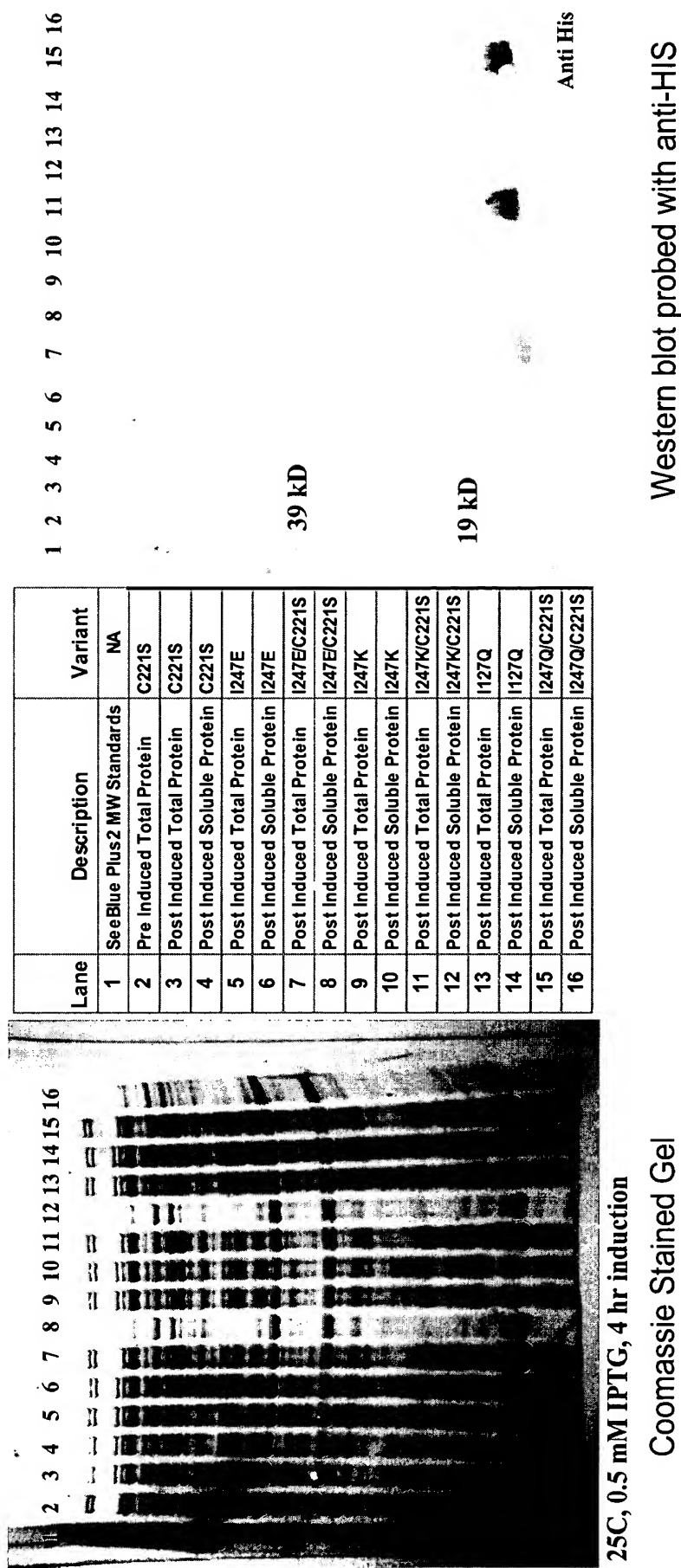
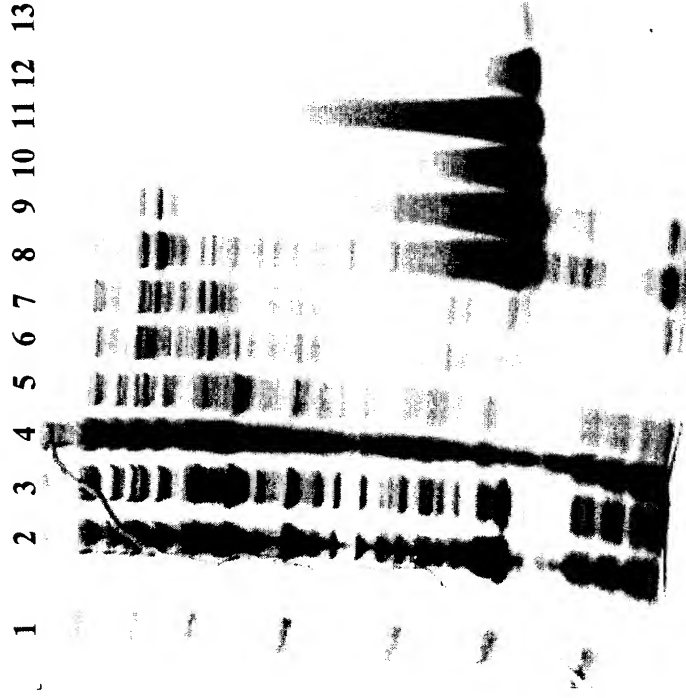


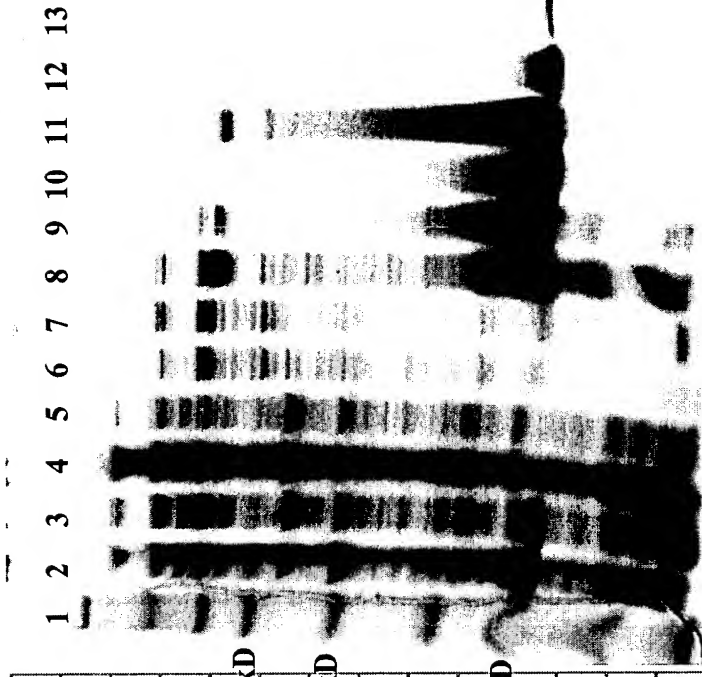
Figure 7

I247E/C221S



25C, 0.5 mM IPTG, 4 hr induction
Coomassie Stained Gel

I247D/C221S



Coomassie Stained Gel

Figure 8

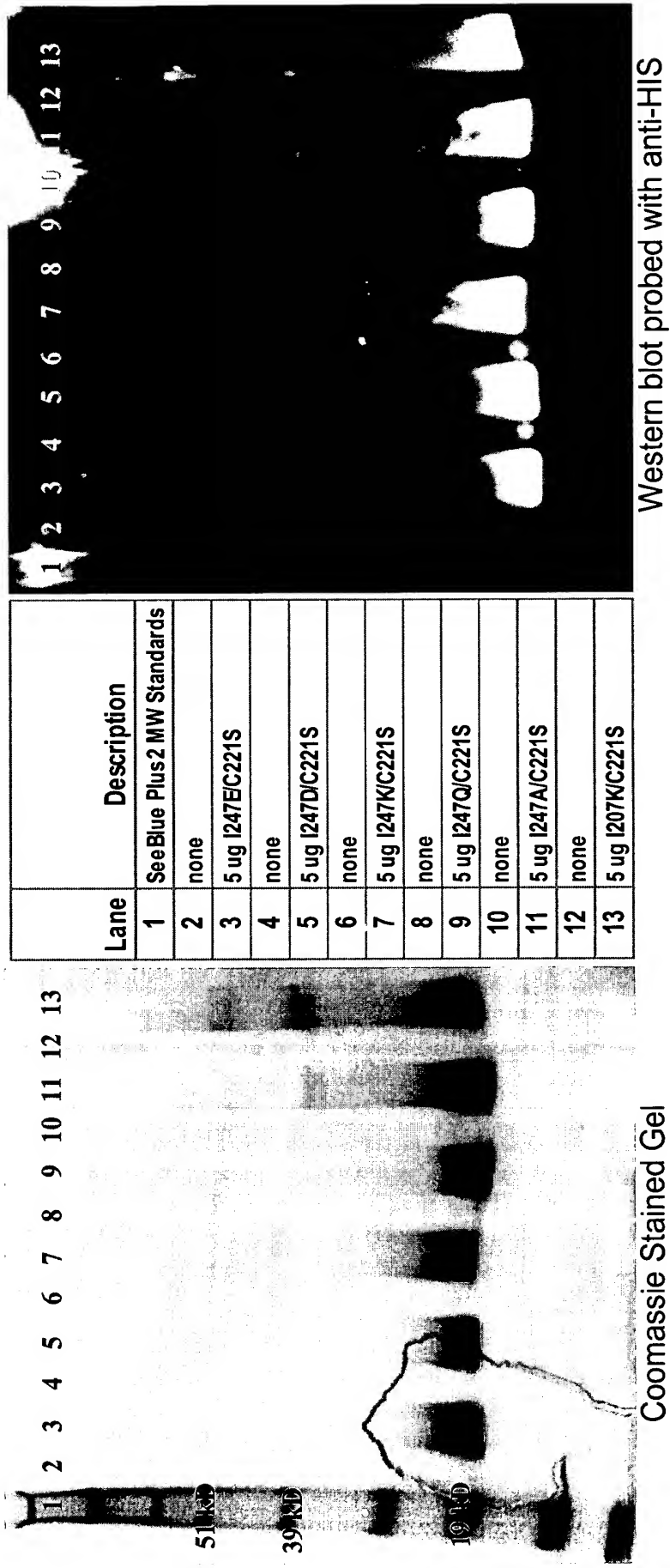


Figure 9

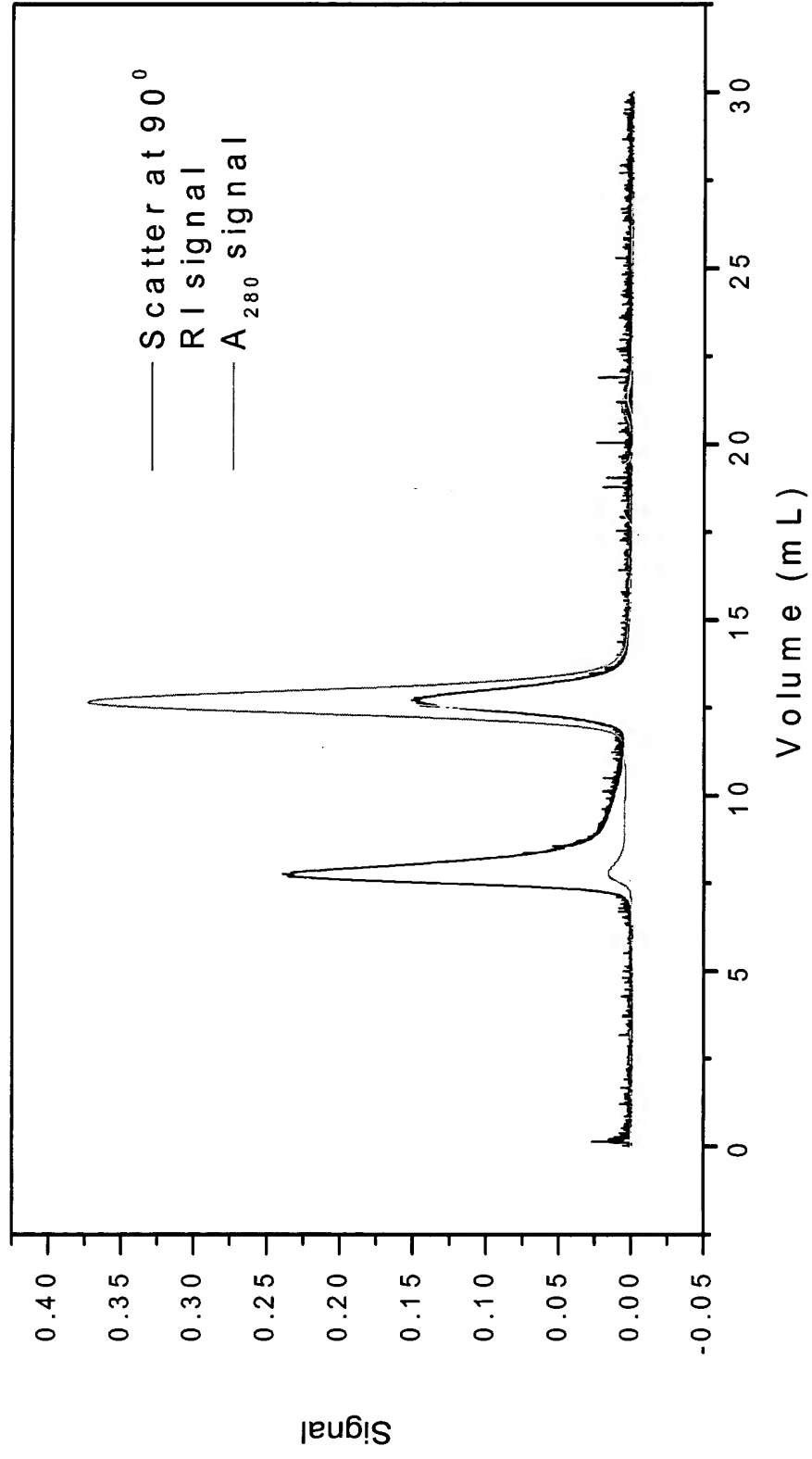
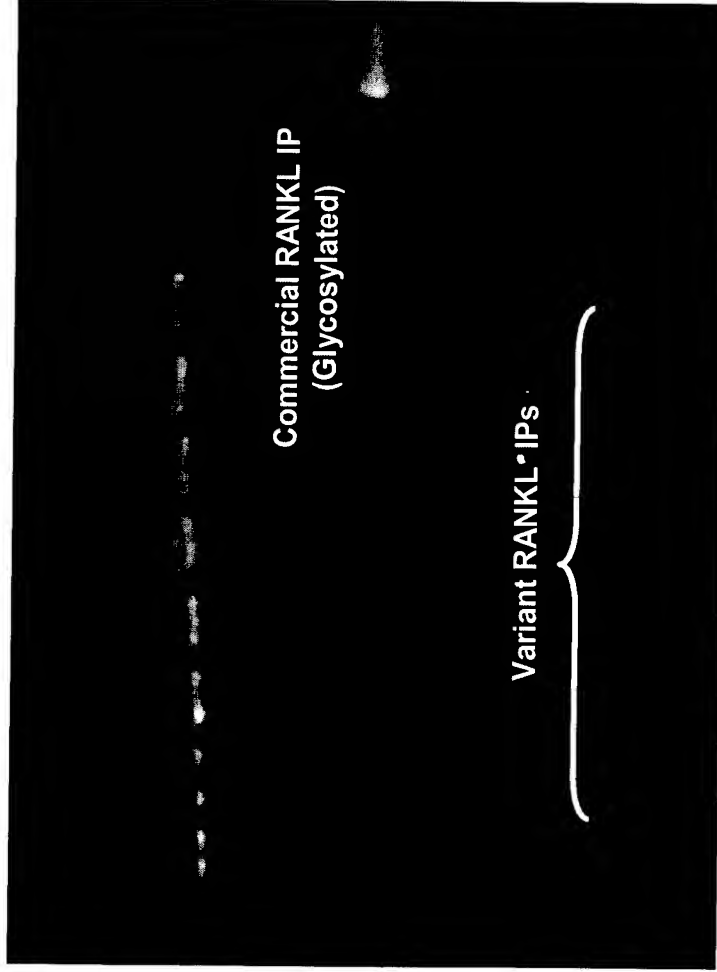


Figure 10

Variant	Mw (Kd)	% Trimer	% Oligomer	Expression
C221S/I247E	65	97	3	1.4 mg/g
C221S/I207K	68	90	10	1.2 mg/g
C221S/I247K	71	84	16	1.2 mg/g
C221S/I247Q	69	91	9	1.1 mg/g
C221S/I247A	67	92	8	1.4 mg/g
C221S/I247D	68	92	8	1.3 mg/g

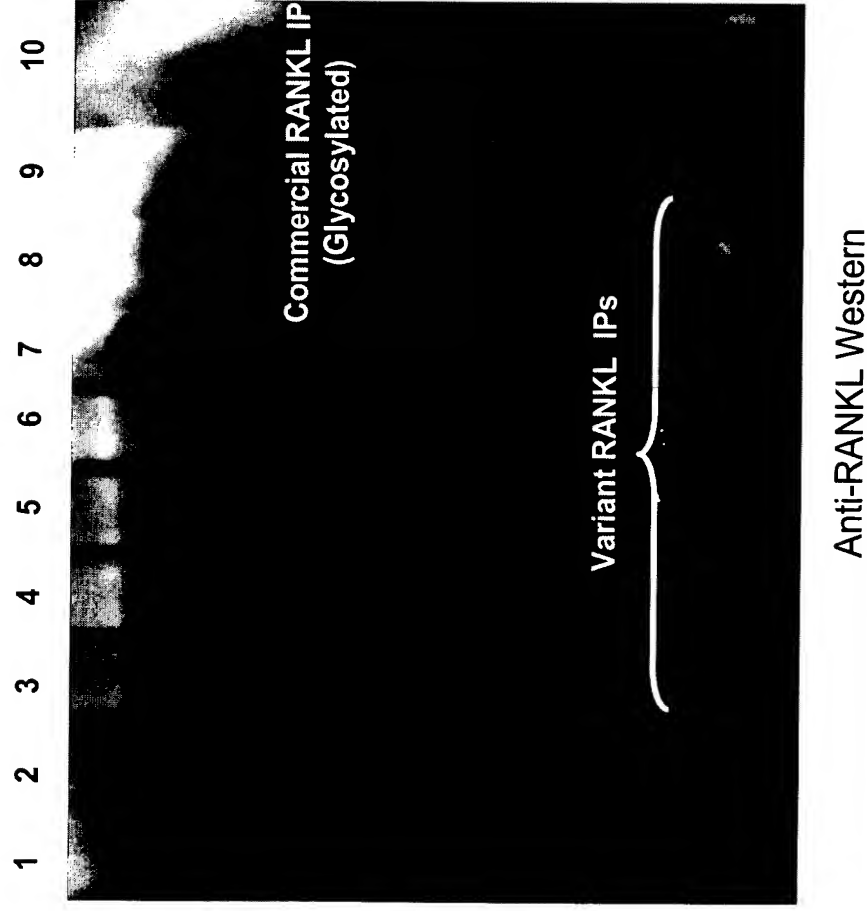
Figure 11

1 2 3 4 5 6 7 8 9 10 11 12



Lane	Description
1	C221S/I247E, no RANK-Fc IP
2	no ligand, 1 ug RANK-Fc IP
3	200 ng C221S/I247E, 1 ug RANK-Fc IP
4	200 ng C221S/I247D, 1 ug RANK-Fc IP
5	200 ng C221S/I247K, 1 ug RANK-Fc IP
6	200 ng C221S/I247Q, 1 ug RANK-Fc IP
7	200 ng C221S/I247A, 1 ug RANK-Fc IP
8	200 ng C221S/I207K, 1 ug RANK-Fc IP
9	200 ng R&D RANKL, 1 ug RANK-Fc IP
10	none
11	0.5 ug C221S/I247E
12	0.5 ug R&D RANKL

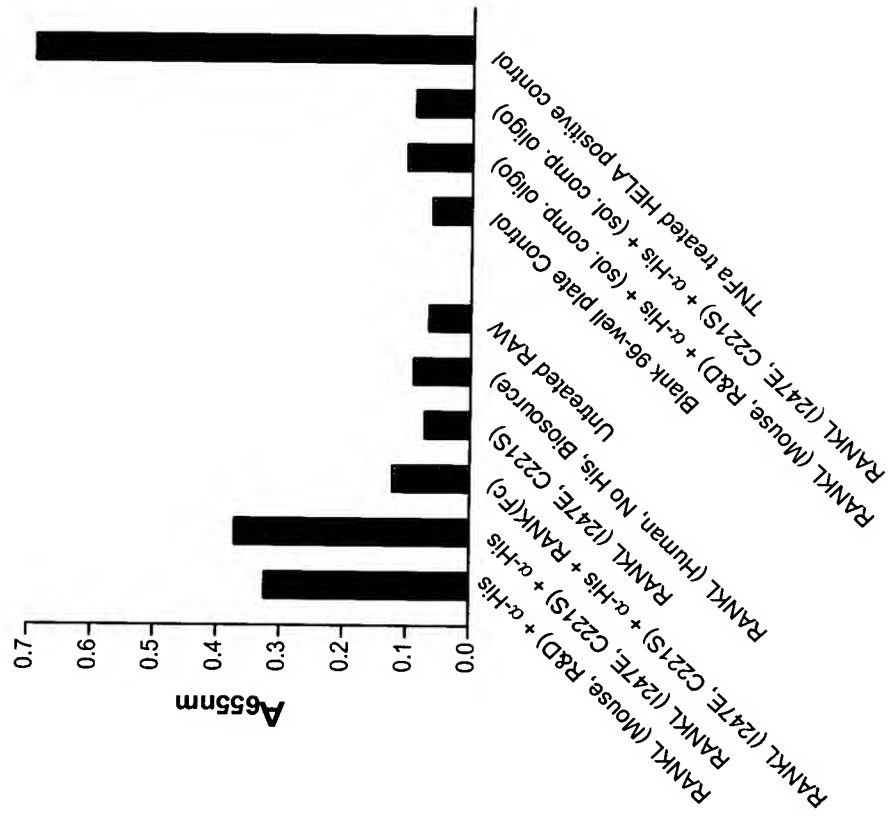
Figure 12



Lane	Description
1	no receptor, 200 ng C221S/I247E
2	1ug OPG/Fc, no ligand
3	1ug OPG/Fc, 200 ng C221S/I247E
4	1ug OPG/Fc, 200 ng C221S/I247D
5	1ug OPG/Fc, 200 ng C221S/I247K
6	1ug OPG/Fc, 200 ng C221S/I247Q
7	1ug OPG/Fc, 200 ng C221S/I247A
8	1ug OPG/Fc, 200 ng C221S/I207K
9	1ug OPG/Fc, 100 ng R&D RANKL
10	500 ng C221S/I247E

Figure 13

Figure 14



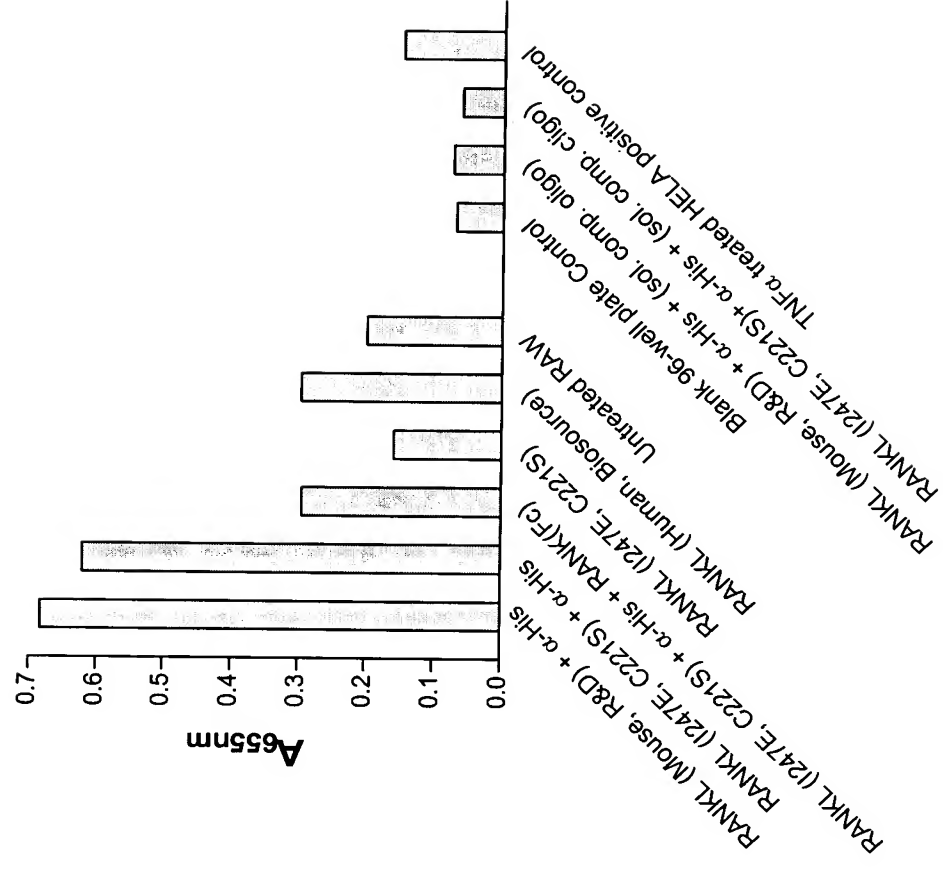
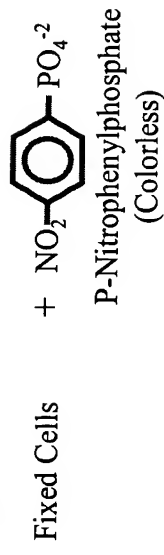
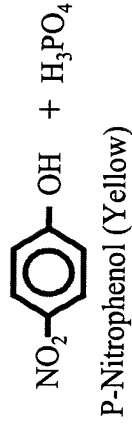


Figure 15

TRAP Solution Assay



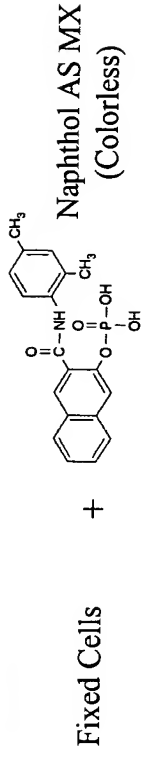
Cellular
Phosphatase
NaTartrate,
pH 5.0



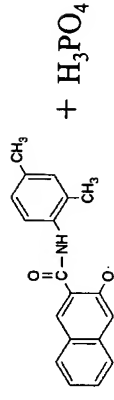
Measure absorbance at 405 nm

Figure 16

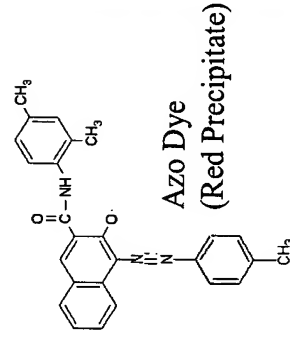
TRAP Staining Assay



Cellular
Phosphatase
NaTartrate,
pH 5.0



Diazonium Salt
(Fast Red Violet LB)



Stained Osteoclasts

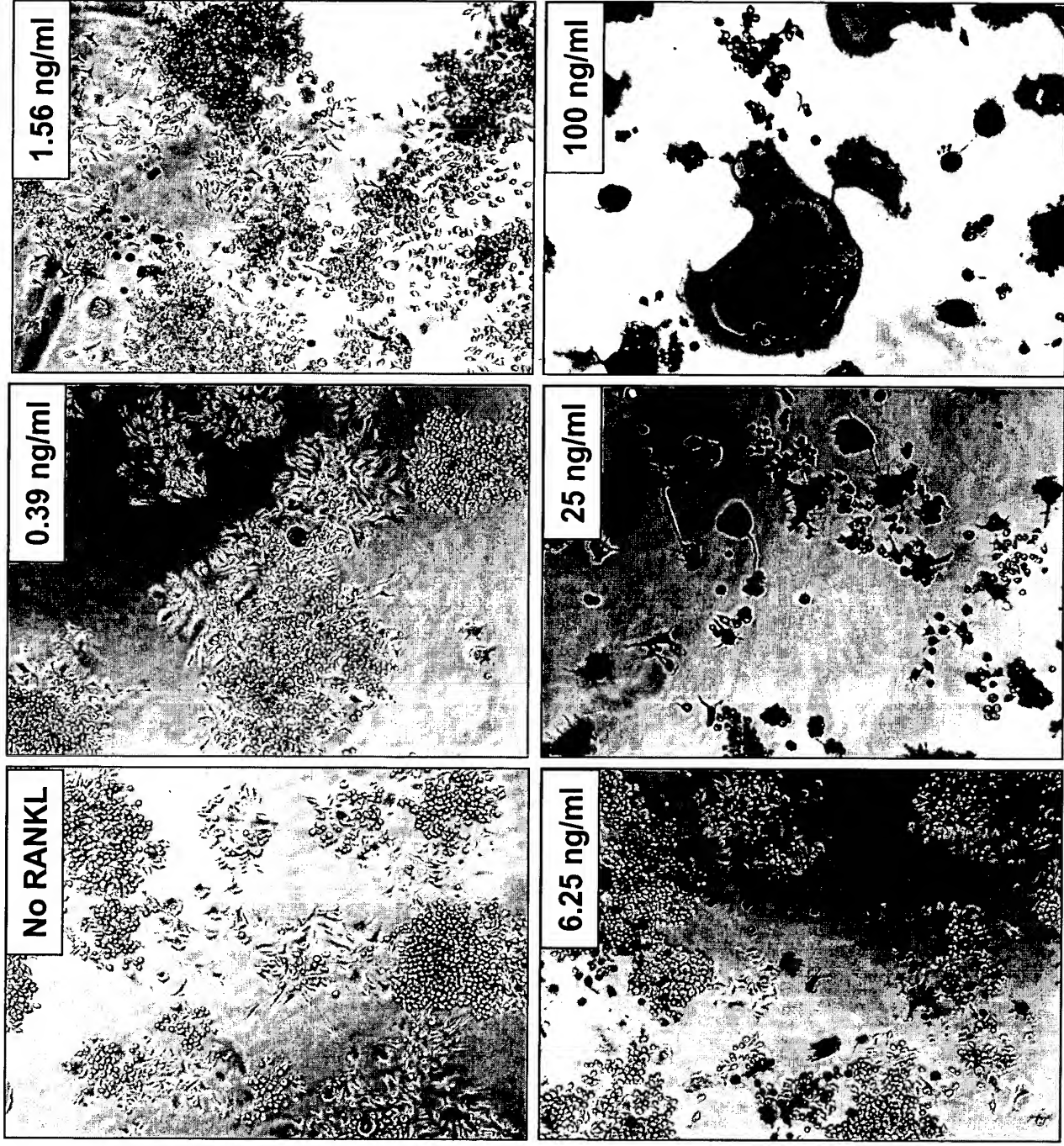


Figure 17

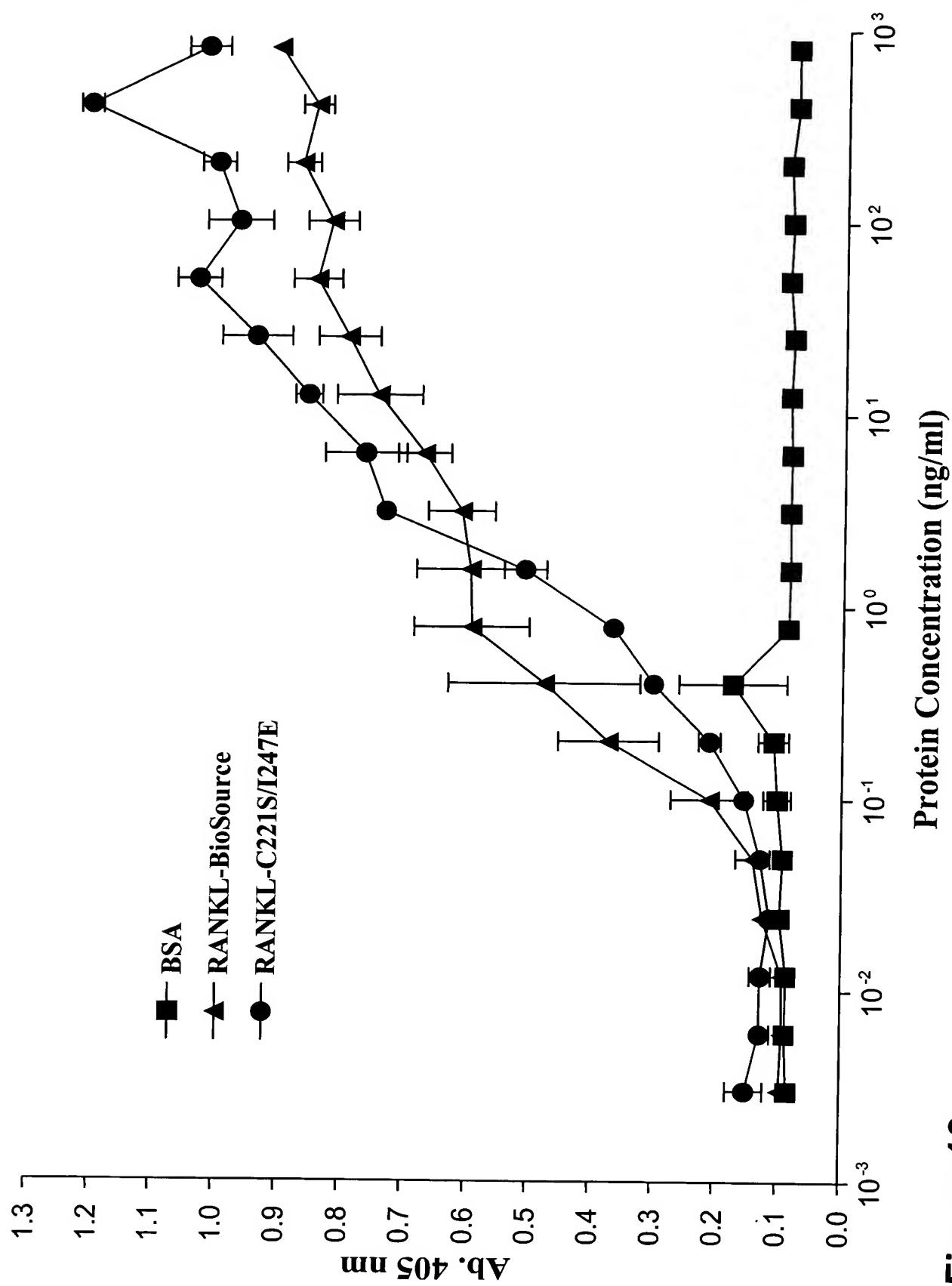


Figure 18

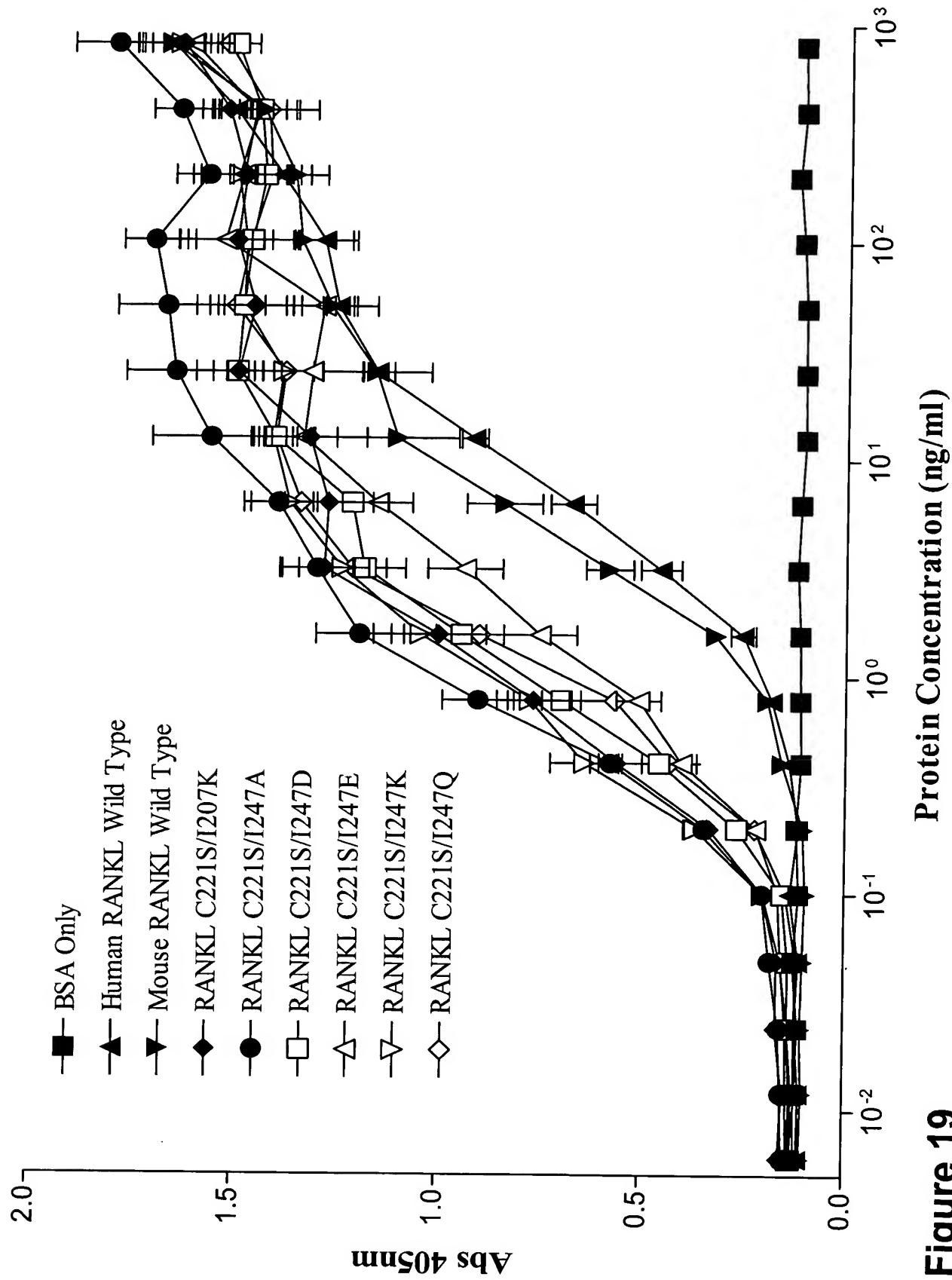


Figure 19

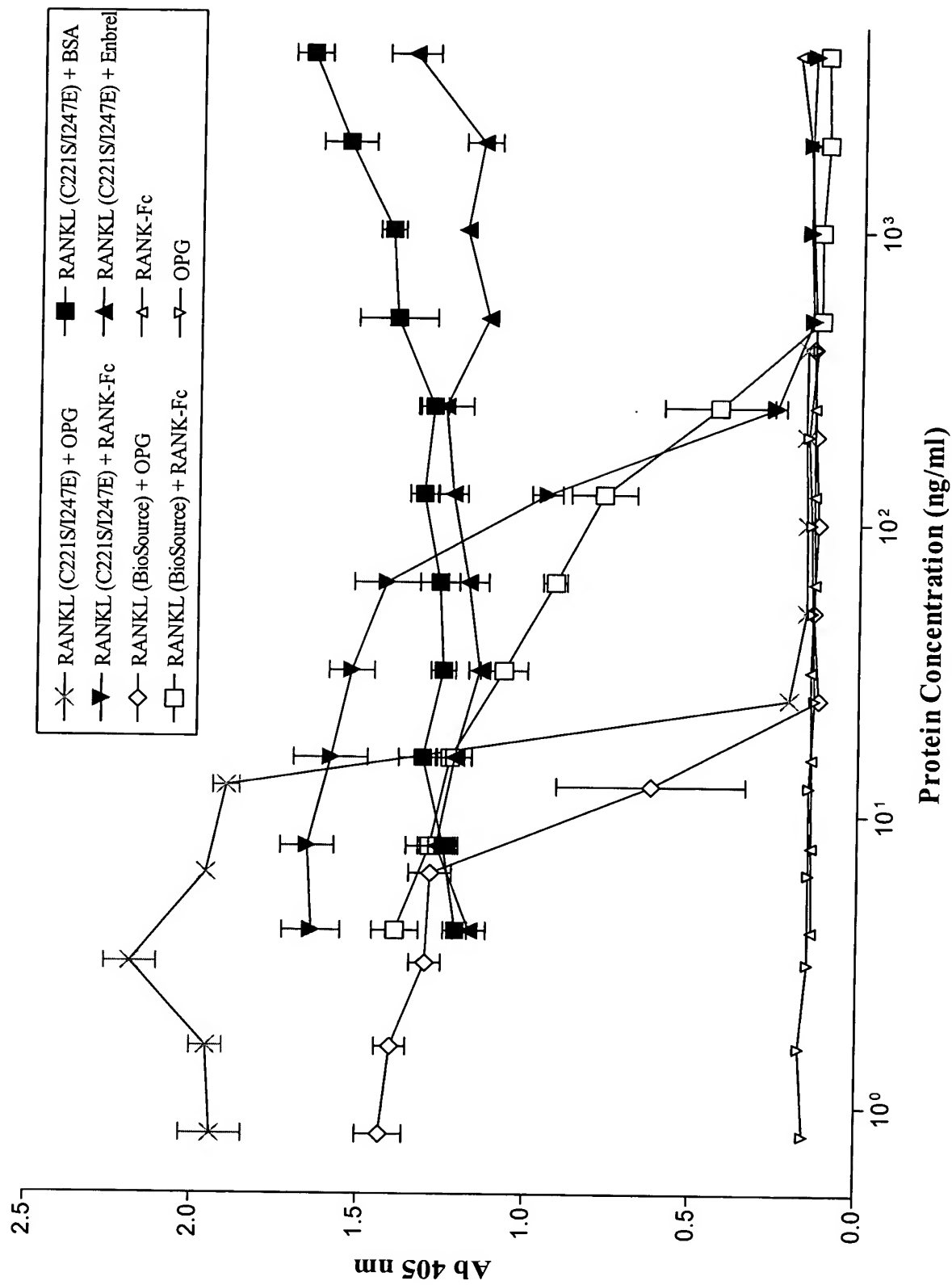


Figure 20

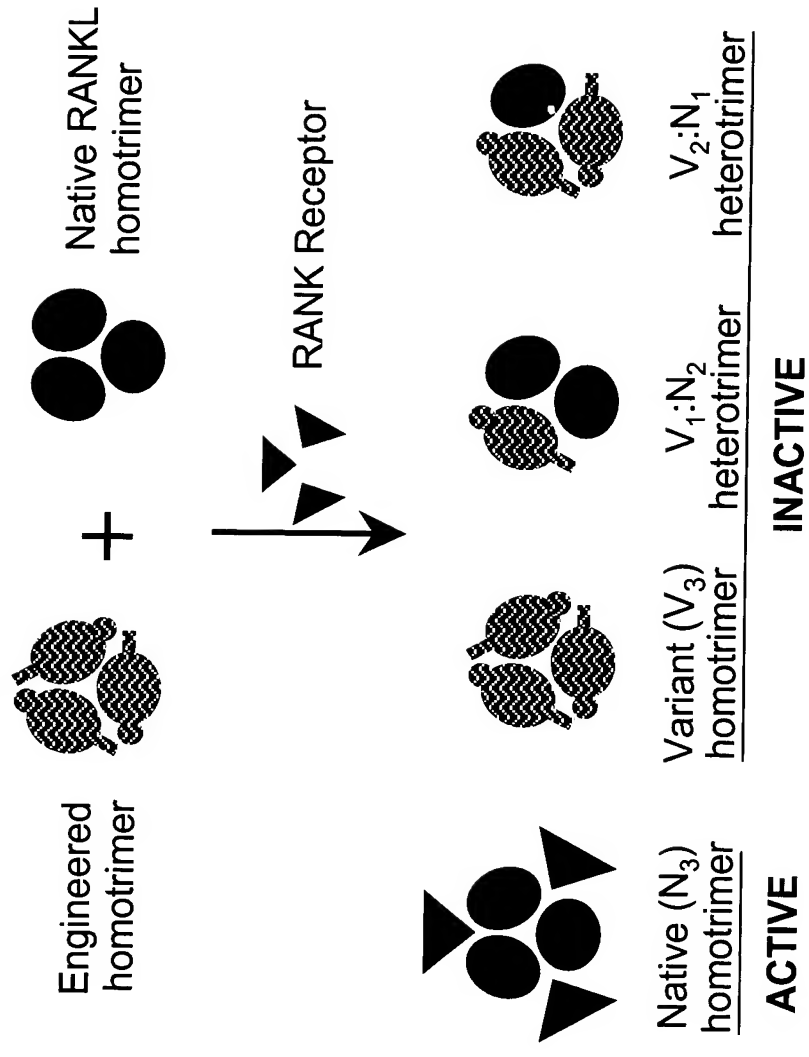


Figure 21

Single Chain Dominant Negative Polypeptides

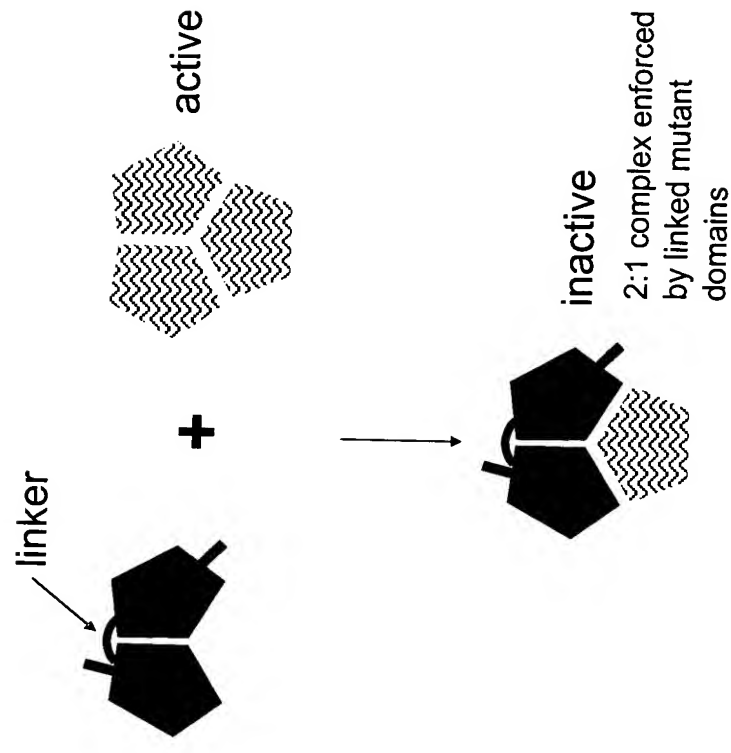


Figure 22

A172R	G191A	I249R	D190T-K248E
A172Q	R223Q	H253T	H225R-I249R
S179E	R223E	H253S	H225N-I249R
S179D	R223M	S268D	E226Q-I249R
H180E	H225R	E269R	E226R-I249R
H180N	H225N	E269Q	H225R-G192A
H180S	H225T	E269T	H225N-G192A
K181Q	H225E	E269K	H225R-D190Q
K181E	E226Q	F270V	H225R-D190T
K181R	E226R	F270E	E269K-F270T
S183K	E226D	F270T	E269T-F270T
S183E	T227K	F270K	H225E-E269K
Y188F	T227Q	R284E	H225E-F270T
Y188M	S228H	S297Q	H225R-E269K
Y188Q	S228E	S297E	H225R-F270T
Y188E	S228D	S297D	I249R-S228H
H189E	T233R	D300N	I249R-S228E
H189A	T233D	D302E	K248E-T227K
H189R	T233E	D302Q	K248E-T227Q
D190Q	Q237K	D302H	G192A-H225R-I249R
D190T	Q237E	R191K-I249R	G192A-H225N-I249R
R191K	Q237T	R191E-I249R	R191K-E226Q-K248E
R191E	K248E	G192A-K248E	R191K-E226R-K248E
R191Q	I249Q	D190Q-K248E	H225E-E226D-F270T

Figure 23

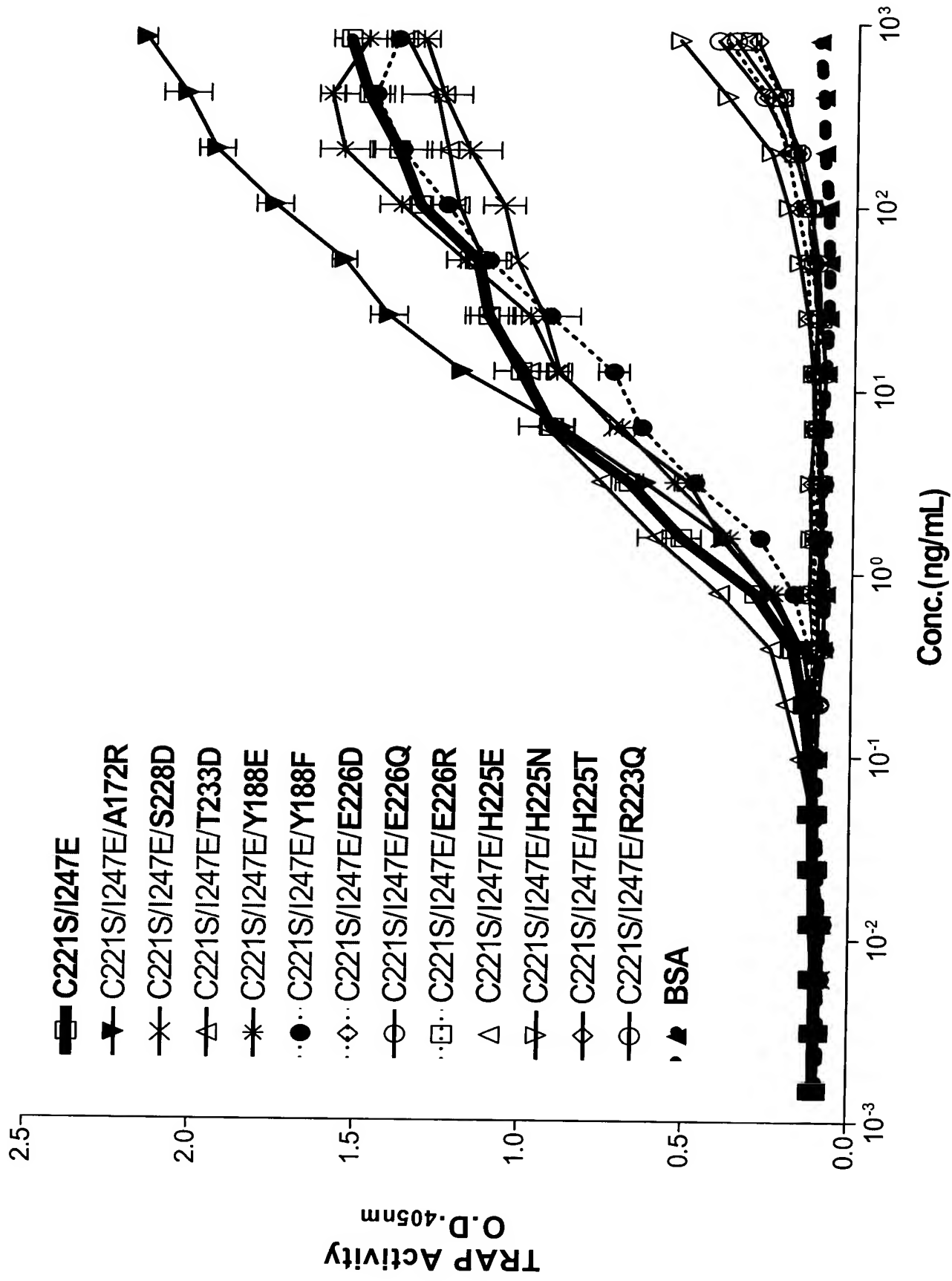


Figure 24

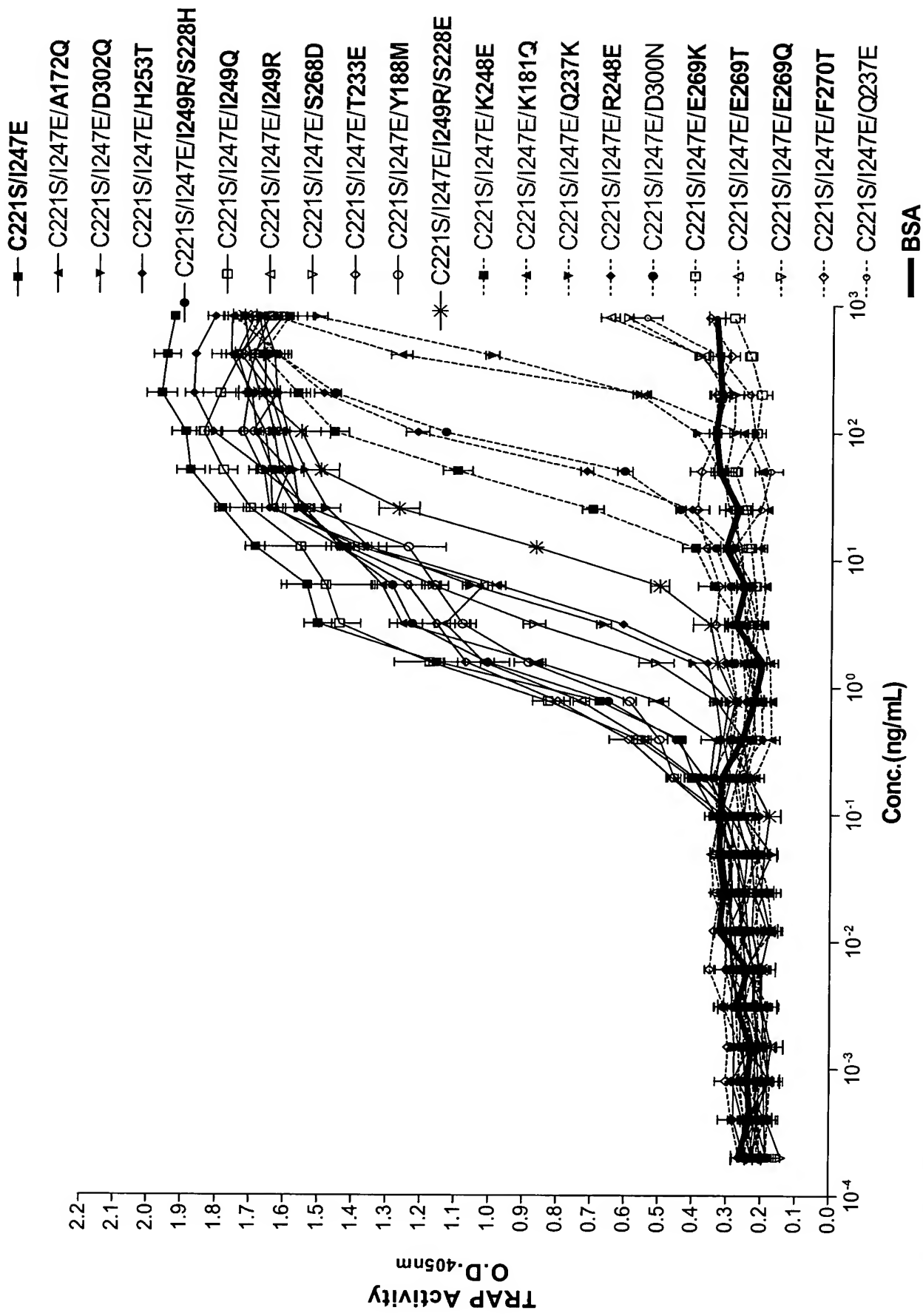


Figure 25

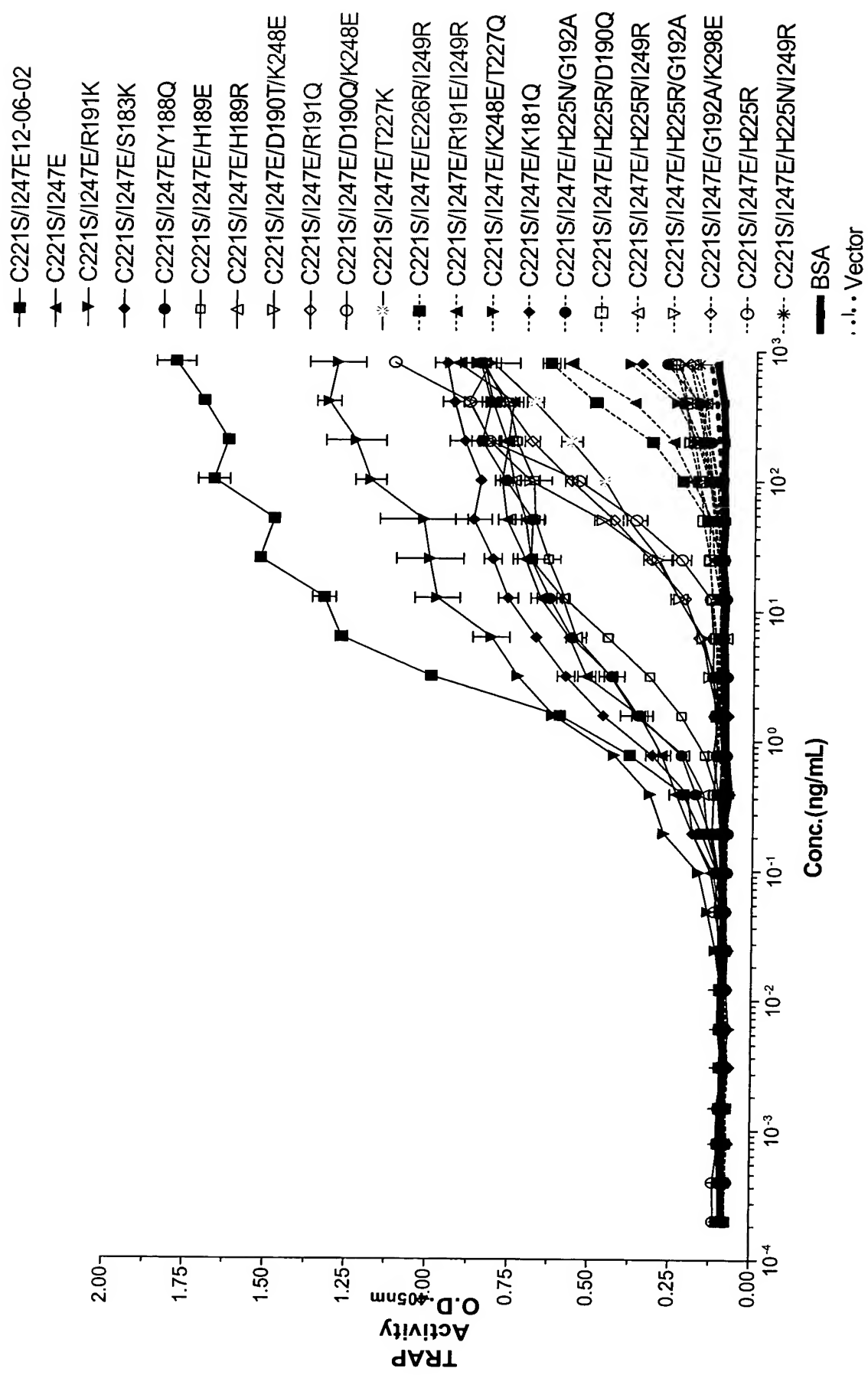


Figure 26

Agonism Assay Using Human PBMCs

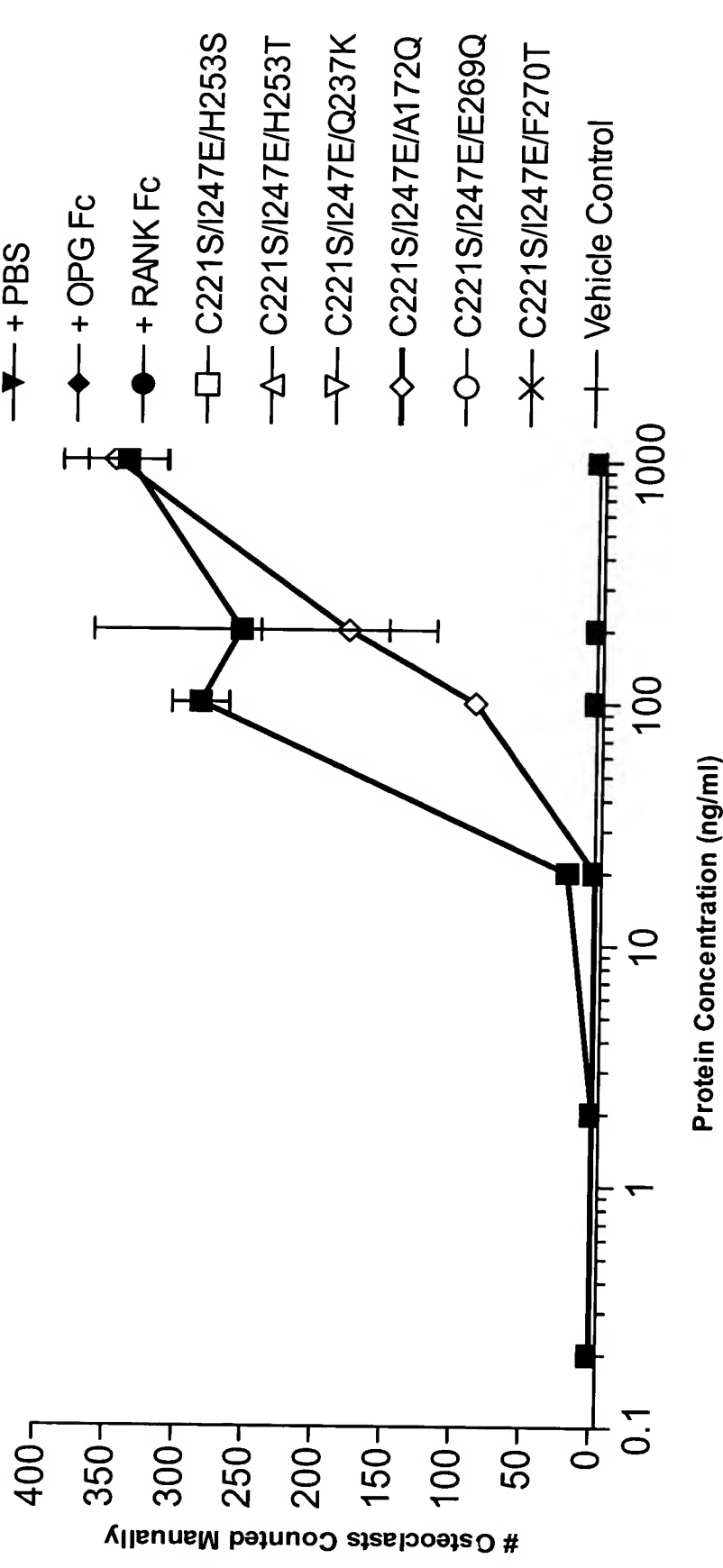


Figure 27

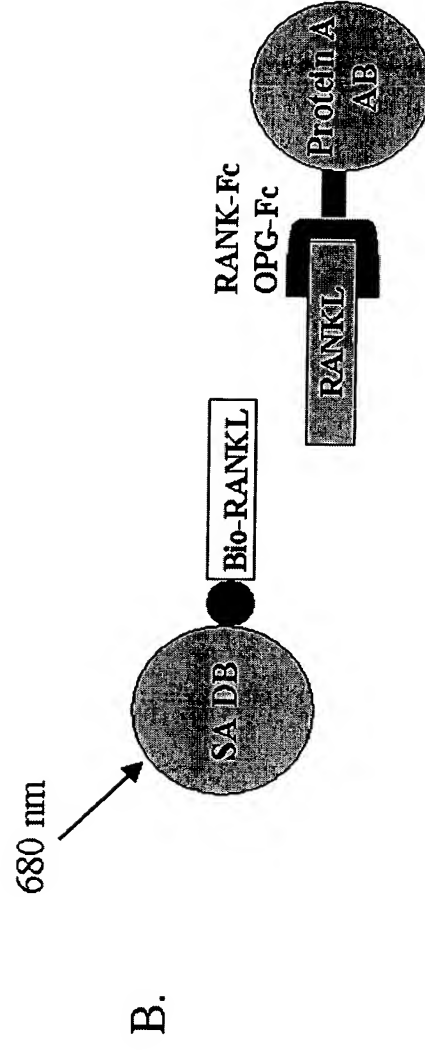
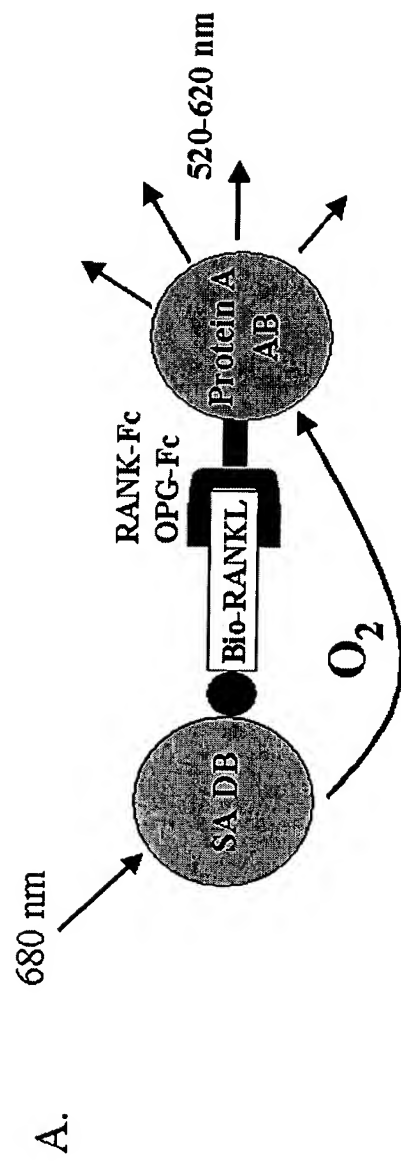
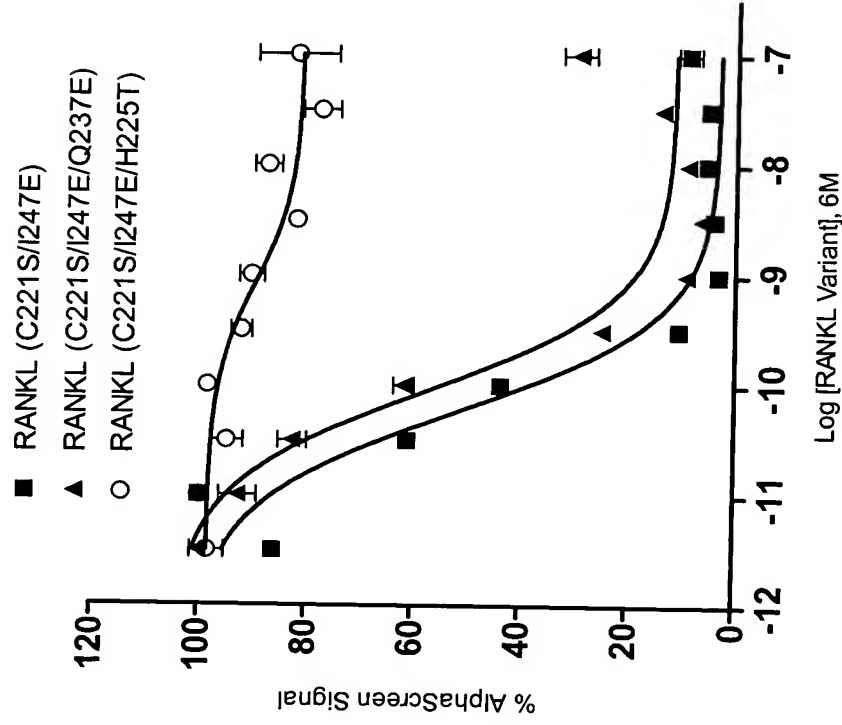


Figure 28

hRANK-Fc Binding Curves



hOPG-Fc Binding Curves

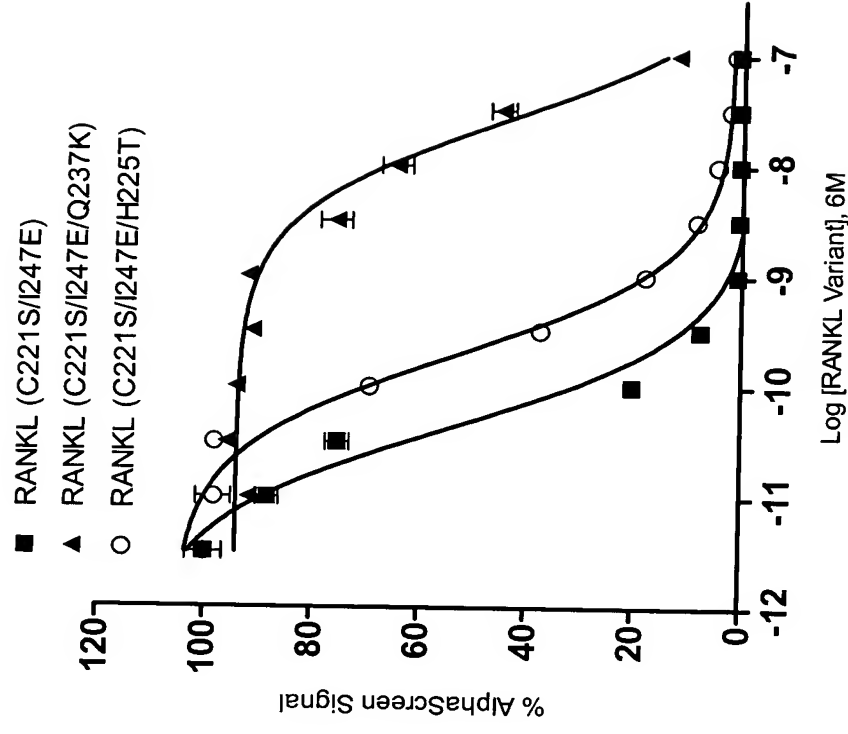


Figure 29

hRANK-Fc Binding Curves

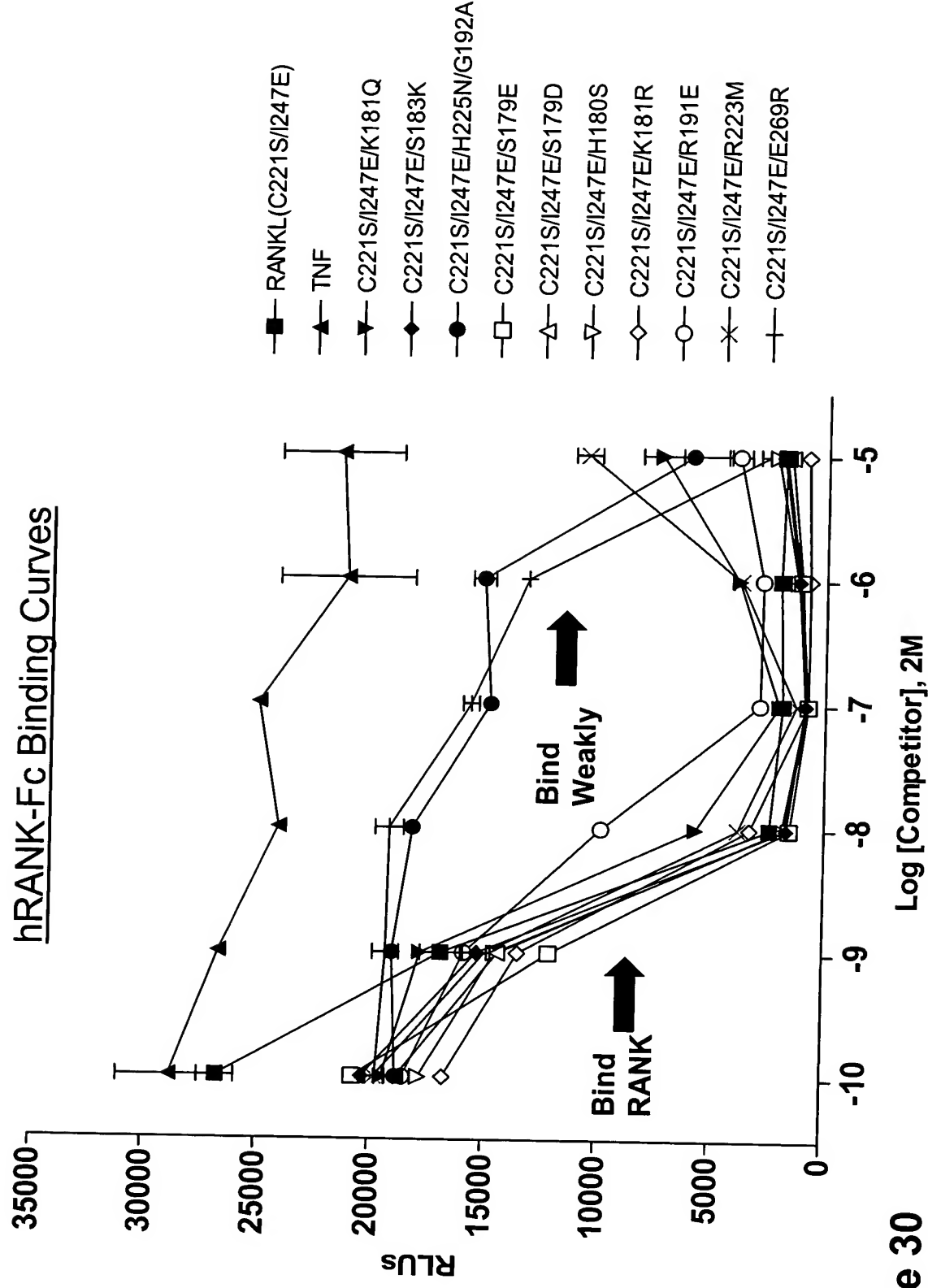


Figure 30

hOPG-Fc Binding Curves

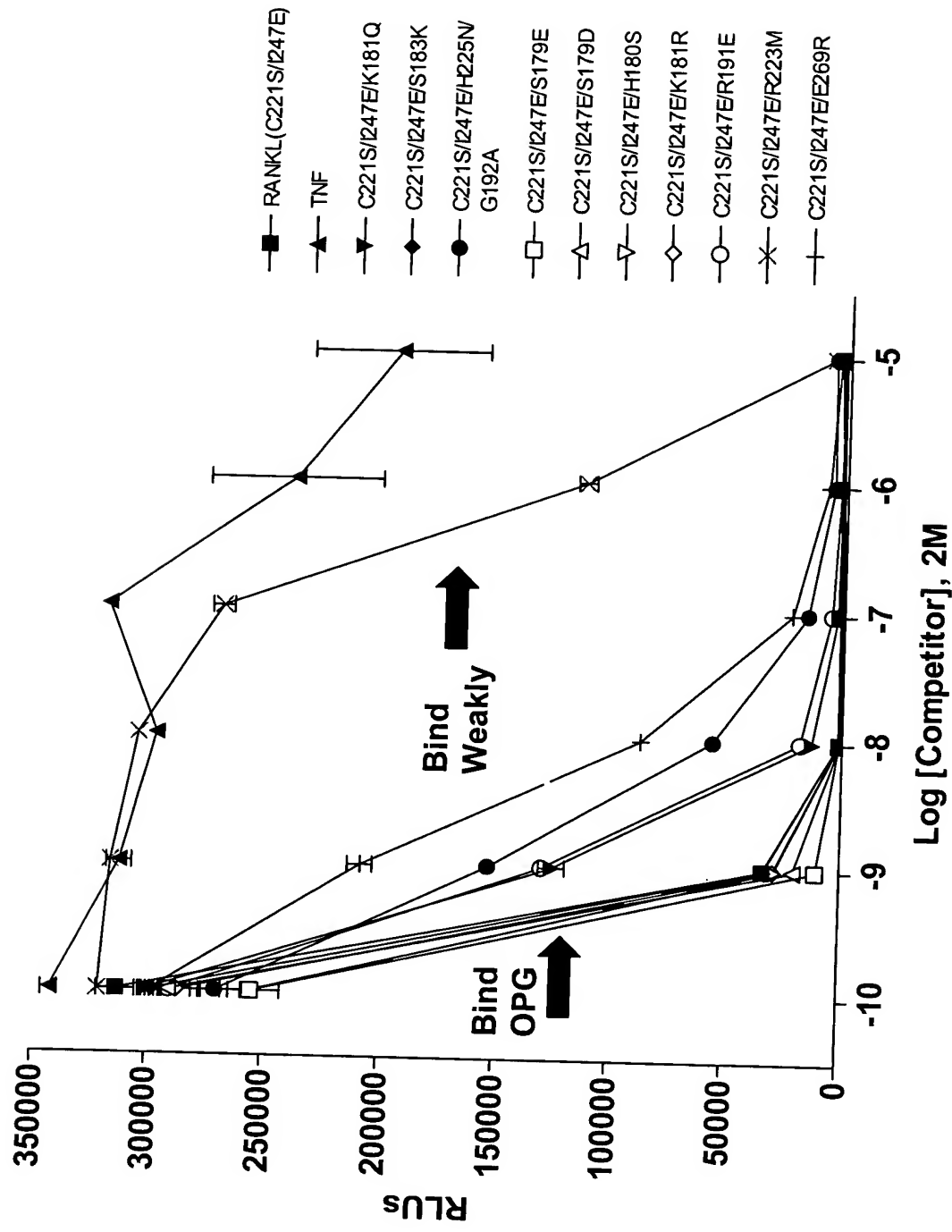


Figure 31